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BRIEF COMMUNICATION

EXPERIMENTAL INFECTION OF SWISS AND AKR/J MICE WITH *Centrocestus formosanus* (TREMATODA: HETEROPHYIDAE)

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SUMMARY

In order to better understand the biology of *Centrocestus formosanus* in a definitive host model, mice of Swiss and AKR/J strains were experimentally infected with 100 metacercariae of the parasite. Fourteen days post-infection, the rodents were killed and adult trematodes were recovered from the small intestine. The percentage of parasite recovery from AKR/J mice (11.4%) was significantly higher than that from Swiss mice (5.3%). Moreover, trematodes recovered from the AKR/J strain were more developed and had greater fecundity. Peculiarities concerning the mice's immune system could explain the difference in susceptibility and in worm development seen in the present study. The data obtained confirm that mice are susceptible to infection with *C. formosanus* and indicate that the AKR/J strain provides a more favorable environment for parasite development.

KEYWORDS: Foodborne intestinal flukes; Trematodes; Experimental models; Heterophyids.

The use of rodents as experimental models for some human parasites has led to a better understanding of trematode biology, but important differences in particular host-parasite relationships can be observed, depending on the strains of the definitive host, especially in the case of mice[6,7,16,22]. Nevertheless, there is a lack of knowledge regarding experimental definitive hosts of some human intestinal trematodes. In fact, over 70 species of foodborne flukes have already been correlated with human infection[34], but many of them have not been studied at length, and these include some heterophyid species.

Heterophyidae are the smallest trematodes that inhabit the intestine of birds and mammals, and the infection of human beings with these flukes has been considered an emerging parasitic disease usually acquired through the consumption of raw or undercooked fish. *Heterophyes heterophyes* and *Metagonimus yokogawai*, the two heterophyid species that most affect humans, are common in Africa, Asia and Eastern Europe[8,13]. In Brazil, human heterophyiasis by *Ascocotyle (Phagicola) longa* has already been reported[1,10,11].

Additional details about the life history of the less prevalent species of heterophyids in vertebrate models are desirable. *Centrocestus formosanus* is an Asian species that has now been introduced to Brazil[30], and although it has also been identified in humans in Asia[8,36], the quantitative aspects of experimental infection of this parasite in mice are unknown. Furthermore, KIMURA *et al.* (2007)[21] suggested that species of *Centrocestus* can be used to study human pathogenic heterophyids, since it has advantages over *Metagonimus* species. These authors argued that it is easier to obtain *Centrocestus* because of its widespread distribution and its relatively short development time in the host (three days), and in addition, rats and hamsters have been reported to be susceptible hosts.

Therefore, knowledge of the biology of the parasite in mice may be interesting not only intrinsically, but also because *C. formosanus* may constitute an experimental model for other parasites.

Over recent years, the inbred AKR strain of mouse and its sublineages have been acquiring greater importance for evaluating the biology of intestinal nematodes[5,12,15,17,19,24,25,28,35]. However, studies on trematodes using the AKR strain remain scarce and address experimental infection using the species *Brachylaima cribbi*[6], *Schistosoma mansoni*[4,15,27] and *Zygocotyle lunata*[2]. The permissiveness of AKR/J mice towards some of these helminths has been correlated to their immunological peculiarities such as deficiency in the complement pathway[3,4], polarization of the T helper cell (Th)-1 response[12], and a less substantial increase in the number of goblet cells associated with mucin deficiency in mucosa during experimental intestinal infection[17].

In order to better understand the biology of *C. formosanus* in a rodent definitive host model, the susceptibility of both Swiss and AKR/J mice

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to the parasite and the potential of these animals as experimental models were assessed.

Metacercariae of *C. formosanus* were obtained from naturally infected *Astraloheros facetus* that were caught from the Pampulha reservoir, an artificial lake located in the city of Belo Horizonte, Minas Gerais, Brazil (19°51'771"S, 43°58'542"W). The gills of these fish were removed, transferred to glass slides containing saline solution (0.85% NaCl) and analyzed under a stereomicroscope. The tissue samples containing metacercariae of the parasite were immersed in a digestion solution (0.85% NaCl in distilled water with 1% pepsin and 1% HCl, pH 2) for one hour at 37 °C. The metacercariae of *C. formosanus* were then collected and counted using a light microscope.

Swiss mice (25 ± 2 g; n = 12 females) and AKR/J mice (25 ± 2 g; n = 18 females) maintained in laboratory conditions and provided with food and water *ad libitum* were used as the experimental definitive hosts. Each animal received an oral suspension containing 100 metacercariae of *C. formosanus*. All rodents were killed by cervical dislocation, 14 days post-infection (DPI), in accordance with the local animal experimentation ethics committee (CETEA/UFMG). The small intestines had been excised and opened longitudinally using scissors in Petri dishes containing saline solution. Trematodes obtained from each rodent were killed in hot water (70 °C), fixed in 10% formalin solution, stained with acetic-alum carmine, cleared in beechwood creosote and mounted in Canada balsam.

The parasites from each strain of mice were analyzed and classified according to their sexual development (i.e. mature flukes present vitellaria and eggs in the uterus, while immature ones do not), and their intrauterine eggs were counted under a light microscope. The measurements of length and width of the body, oral sucker, acetabulum, pharynx, ovary, and testes were taken with the aid of an ocular micrometer fitted to a light microscope. The total area of the worms was measured by means of the AxioVision LE software (release 4.8.2) following image capture from a digital camera attached to a microscope (Leica DM500).

The data obtained were subjected to a Student's t test for means, and to the χ^2 test or Fisher's exact test for proportion comparisons.

Both strains of mice were susceptible to the parasite, but a significantly greater number of parasites (p = 0.006) were recovered from AKR/J mice (11.4 ± 6.2) in comparison to those from Swiss mice (5.3 ± 4.5). In the inbred strain, the worms were more developed, such that they more commonly presented vitellaria and had many more eggs in the uterus. In fact, 84.4% of flukes recovered from AKR/J mice were mature while this value in Swiss was 67.9%, with a significant difference (p = 0.007). The percentages of animals which tested positive after oral administration of metacercariae were 83.3 and 94.4%, for Swiss and AKR/J strains of mice respectively (Table 1; Fig. 1).

There is still no completely clear information on which vertebrates might be suitable hosts for *C. formosanus*. There are scattered reports in literature, mostly only of a qualitative nature, on infection of various hosts such as cats, rats, mice, hamsters, pigeons, chickens and ducks with this heterophyid, at different times of infection[9,18,23,29-31,33]. However, rats and cats have been considered to be more favorable experimental hosts[9,23].

Table 1
Measurements of specimens of *Centrocestus formosanus* recovered from Swiss and AKR/J mice at 14 days post-infection. Measurements are presented in micrometers (μm), except for the area (μm²). Abbreviations: A = area; L = length, W = width, N = number, NS = not-significant.

Parasites		Swiss mice (n = 30)	AKR/J mice (n = 60)	p-value
Body	L	345 ± 23	334 ± 29	NS
	W	127 ± 17	143 ± 18	0.0001
	A	34743 ± 4929	36569 ± 5596	NS
Oral sucker	L	49 ± 5	49 ± 4	NS
	W	44 ± 3	48 ± 4	0.0001
Acetabulum	L	40 ± 5	44 ± 4	0.0012
	W	37 ± 4	39 ± 4	0.0136
Pharynx	L	34 ± 3	36 ± 4	NS
	W	20 ± 2	21 ± 3	NS
Ovary	L	37 ± 6	36 ± 7	NS
	W	27 ± 3	26 ± 5	NS
Testes	L	48 ± 7	48 ± 7	NS
	W	29 ± 4	30 ± 5	NS
Intrauterine eggs	N	5 ± 6	10 ± 9	0.0289

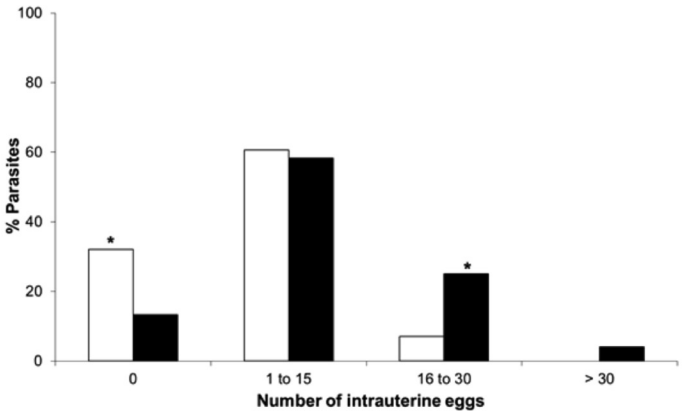

Fig. 1 - Number of intrauterine eggs in *Centrocestus formosanus* recovered from Swiss (white column) and AKR/J mice (black column) at 14 days post-infection. Asterisks indicate a significant increase ($p < 0.05$).

The respective mean recovery percentages of *C. formosanus* from Swiss and AKR/J strains of mice (5.3 and 11.4%, respectively) are consistent with the recovery rates obtained by CHEN (1942)[9] using three mice that were individually infected with different numbers of metacercariae and were evaluated at different time periods after infection. In the present study, the mean recovery percentage of *C. formosanus* from mice is shown for the first time, as well as the ratio between immature and mature worms, for two different strains of mice.

The findings of the present study suggest that AKR/J mice provide a more favorable environment for parasite development. Regarding the size of the worms, those from AKR/J mice were significantly wider than

those from Swiss mice, but the length and the total area did not change. The AKR/J strain also seemed to have enabled further development of the suckers of the parasite, or perhaps they were preserved for a longer period of time. In experimental infection of hamsters with *C. formosanus*, the suckers degenerated with advancing infection, and it was suggested that these alterations could be related to the elimination of parasites from the intestine of the definitive host[33].

In addition to the differences in the mean number of parasites, differences in morphology and sexual development were observed between the *C. formosanus* recovered from Swiss and AKR/J mice, which may indicate a phenomenon of phenotypic plasticity. It is possible that the immunological characteristics of the AKR/J strain are related to this finding. In fact, the fecundity of helminths seems to be directly linked to the host immune system and may be influenced by immunological variations correlated with strains of mice[4,22], immunomodulatory drugs[20,26] and experimental co-infection[5,15].

The role of intestinal goblet cells in the expulsion of the trematodes *Echinostoma trivolvis*[14] and *Gymnophalloides seoi*[16,32] has been demonstrated in other strains of mice, and mastocytosis and goblet cell hyperplasia with mucin activation were observed as local immune responses against *Neodiplostomum seoulense*[7].

Therefore, the immunological characteristics of AKR mice already mentioned[3,12], and in particular the peculiarities of their goblet cells and intestinal mucins[17], can be related to their greater susceptibility to *C. formosanus*. However, further studies on these immunopathological characteristics of the infection of AKR/J mice with this trematode are still needed.

Our data are complementary to existing information regarding the experimental model for heterophyids, therefore confirming that mice, and in particular the AKR/J strain, are susceptible and may be a promising model for studying infection with *C. formosanus*, and perhaps with other intestinal flukes. The factors directly related to higher susceptibility of mice of this inbred strain to the parasite remain unknown.

RESUMO

Infecção experimental de camundongos Swiss e AKR/J por *Centrocestus formosanus*(Trematoda: Heterophyidae)

Com o objetivo de melhor conhecer a biologia de *Centrocestus formosanus* em um modelo de hospedeiro definitivo, camundongos das linhagens Swiss e AKR/J foram experimentalmente infectados com 100 metacercárias do parasito. Quatorze dias após a infecção, os roedores foram mortos e os trematódeos adultos recuperados do intestino delgado. O percentual de recuperação do parasito de camundongos AKR/J (11,4%) foi significativamente maior que o obtido de camundongos Swiss (5,3%). Além disso, trematódeos recuperados da linhagem AKR/J apresentavam-se mais desenvolvidos e apresentaram maior fecundidade. Peculiaridades relacionadas ao sistema imunológico dos camundongos podem explicar as diferenças observadas na suscetibilidade e no desenvolvimento dos vermes no presente estudo. Os dados obtidos confirmam que camundongos são suscetíveis à infecção pelo *C. formosanus* e indicam que a linhagem AKR/J proporciona um ambiente mais favorável para o desenvolvimento do parasito.

REFERENCES

1. Almeida-Dias ER, Woiciechovski E. Ocorrência da *Phagicola longa* (Trematoda: Heterophyidae) em mugilídeos e no homem, em Registro e Cananéia, SP. Hig Alim. 1994;8:43-6.

2. Barbosa FS, Pinto HA, Melo AL. *Biomphalaria straminea* (Mollusca: Planorbidae) como hospedeiro intermediário de *Zygocotyle lunata* (Trematoda: Zygocotylidae) no Brasil. Neotrop Helminthol. 2011;5:241-6.

3. Ben-Efraim S, Cinader B. The role of complement in the passive cutaneous reaction of mice. J Exp Med. 1964;120:925-42.

4. Bicalho RS, Melo AL, Pereira LH. Desenvolvimento do *Schistosoma mansoni* na cavidade peritoneal de camundongos da linhagem AKR/J. Rev Inst Med Trop Sao Paulo. 1993;35:411-6.

5. Bickle QD, Solum J, Helmby H. Chronic intestinal nematode infection exacerbates experimental *Schistosoma mansoni* infection. Infect Immun. 2008;76:5802-9.

6. Butcher AR, Palethorpe HM, Grove DI. The susceptibility of inbred mice to infection with *Brachylaima cribbi* (Digenea: Brachylaimidae). Parasitol Int. 2002;51:109-15.

7. Chai JY, Kim TK, Cho WH, Seo M, Kook J, Guk SM, *et al.* Intestinal mastocytosis and goblet cell hyperplasia in BALB/c and C3H mice infected with *Neodiplostomum seoulense*. Korean J Parasitol. 1998;36:109-19.

8. Chai JY, Shin EH, Lee SH, Rim HJ. Foodborne intestinal flukes in Southeast Asia. Korean J Parasitol. 2009;47(Suppl):69-102.

9. Chen HT. The metacercaria and adult of *Centrocestus formosanus* (Nishigori, 1924) with notes on the natural infection of rats and cats with *C. armatus* (Tanabe, 1922). J Parasitol. 1942;28:285-98.

10. Chieffi PP, Gorla MC, Torres DM, Dias RM, Mangini AC, Monteiro AV, *et al.* Human infection by *Phagicola* sp. (Trematoda, Heterophyidae) in the municipality of Registro, São Paulo State, Brazil. J Trop Med Hyg. 1992;95:346-8.

11. Chieffi PP, Leite OH, Dias RM, Torres DM, Mangini AC. Human parasitism by *Phagicola* sp. (Trematoda, Heterophyidae) in Cananéia, São Paulo State, Brazil. Rev Inst Med Trop Sao Paulo. 1990;32:285-8.

12. Curry AJ, Else KJ, Jones F, Bancroft A, Grencis RK, Dunne DW. Evidence that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected with *Trichuris muris*. J Exp Med. 1995;181:769-74.

13. Fried B, Graczyk TK, Tamang L. Food-borne intestinal trematodiases in humans. Parasitol Res. 2004;93:159-70.

14. Fujino T, Fried B, Tada I. The expulsion of *Echinostoma trivolvis*: worm kinetics and intestinal cytopathology in conventional and congenitally athymic BALB/c mice. Parasitology. 1993;106:297-304.

15. Gazzinelli SEP, Melo AL. Interação entre *Strongyloides venezuelensis* e *Schistosoma mansoni* em camundongos da linhagem AKR/J. Rev Ci Med Biol. 2008;7:149-55.

16. Guk SM, Lee JH, Kim HJ, Kim WH, Shin EH, Chai JY. CD4+ T-cell-dependent goblet cell proliferation and expulsion of *Gymnophalloides seoi* from the intestine of C57BL/6 mice. J Parasitol. 2009;95:581-90.

17. Hasnain SZ, Wang H, Ghia JE, Haq N, Deng Y, Velcich A, *et al.* Mucin gene deficiency in mice impairs host resistance to an enteric parasitic infection. Gastroenterology. 2010;138:1763-71.

18. Hernández LE, Díaz MT, Bashirullah A. Description of different developmental stages of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae). Rev Cient (Maracaibo). 2003;13:285-92.

19. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. J Immunol. 2008;180:2443-9.

20. Juasook A, Boonmars T, Kaewkes S, Loilome W, Veteewuthacharn K, Wu Z, *et al.* Anti-inflammatory effect of prednisolone on the growth of human liver fluke in experimental opisthorchiasis. Parasitol Res. 2012;110:2271-9.

21. Kimura D, Paller VG, Uga S. Development of *Centrocestus armatus* in different final hosts. Vet Parasitol. 2007;146:367-71.

22. Lee KJ, Park SK, Im JA, Kim SK, Kim GH, Kim GY, *et al.* Susceptibility of several strains of mice to *Echinostoma hortense* infection. Korean J Parasitol. 2004;42:51-6.

23. Martin WE. The life histories of some Hawaiian heterophyid trematodes. J Parasitol. 1958;44:305-23.

24. Martins WA, Melo AL, Nicoli JR, Cara DC, Carvalho MA, Lana MA, *et al.* A method of decontaminating *Strongyloides venezuelensis* larvae for the study of strongyloidiasis in germ-free and conventional mice. J Med Microbiol. 2000;49:387-90.

25. Martins WA, Nicoli JR, Farias LM, Carvalho MAR, Cara DC, Melo AL. *Strongyloides venezuelensis*: efeito de antimicrobiano e imunossupressor no curso da infecção em camundongos da linhagem AKR/J. Rev Ci Med Biol. 2009;8:315-24.

26. Mati VLT, Freitas RM. Melo AL. Effects of pentoxifylline during *Schistosoma mansoni* infection in Swiss mice: an analysis of worm burden, fecundity and liver histopathology. J Helminthol. 2010;84:348-54.

27. Mati VLT, Melo AL. Current applications of oogram methodology in experimental schistosomiasis; fecundity of female *Schistosoma mansoni* and egg release in the intestine of AKR/J mice following immunomodulatory treatment with pentoxifylline. J Helminthol. 2012;6:1-10. [Epub ahead of print, DOI: 10.1017/S0022149X12000144]

28. Melo AL, Vasconcelos AC, Gazzinelli SEP. Alterações histopatológicas em camundongos AKR/J reinfectados por *Strongyloides venezuelensis*. Rev Ci Med Biol. 2006;5:7-12.

29. Nath D. Experimental development of *Centrocestus formosanus* (Nishigori, 1924) in Indian domestic poultry with notes on natural infection. Indian J Anim Sci. 1972;42:862-8.

30. Pinto HA, Melo AL. *Melanoides tuberculata* (Mollusca: Thiaridae) as an intermediate host of *Centrocestus formosanus* (Trematoda: Heterophyidae) in Brazil. Rev Inst Med Trop Sao Paulo. 2010;52:207-10.

31. Premvati G, Pande V. On *Centrocestus formosanus* (Nishigori, 1924) Price, 1932 and its experimental infection in white leghorn chicks. Jpn J Parasitol. 1974;23:79-84.

32. Seo M, Guk SM, Han ET, Chai JY. Role of intestinal goblet cells in the expulsion of *Gymnophalloides seoi* from mice. J Parasitol. 2003;89:1080-2.

33. Srisawangwong T, Pinlaor S, Kanla P, Sithithaworn P. *Centrocestus formosanus*: surface morphology of metacercaria, adult and eggs. J Helminthol. 1997;71:345-50.

34. Toledo R, Esteban JG, Fried B. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv Parasitol. 2006;63:285-365.

35. Wahid FN, Behnke JM. Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): parasite specific IgG1 antibody responses and primary response phenotype. Parasite Immunol. 1993;15:401-13.

36. Yu S, Xu L, Jiang Z, Xu S, Han J, Zhu Y, *et al.* Report on the first nationwide survey of the distribution of human parasites in China. 1. Regional distribution of parasite species. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 1994;12:241-7. (Chinese: English summary).

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